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EFFECT OF INTERLEUKINS RESPONSE TO ECM-INDUCED ACQUISITION OF DRUG RESISTANCE IN MCF-7 CELLS

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Aim: **To examine the effect of various components of extracellular matrix (ECM) on acquisition of drug resistance to taxol and camptothecin by breast carcinoma cell line MCF-7.** ***Methods:*** **Cancer cells were cultured on bovine serum albumin (BSA), vitronectin (VN), fibronectin (FN), collagen type I (COL-I), or Matrigel-coated plates with or without taxol (paclitaxel) or camptothecin treatment. The effect of anticancer drugs on cell growth was accessed by XTT assay, and the alterations of cellular morphology were examined by phase contrast microscopy. Immunofluorescence study was performed using monoclonal anti-β-tubulin antibody.** ***Results:*** **All cell lines showed a significant decrease in cell survival when treated with anticancer drugs without components of ECM, whereas survival rates of Caco-2, MCF-7 and NCI-H292 were significantly increased when cells were cultured on COL-I- and Matrigel-coated dishes after treatment with paclitaxel or camptothecin. MCF-7 cells showed and maintained a colony formation when cultured on the COL-I- and Matrigel-coated dish. Moreover, cytotoxicity (IC_{50}) was decreased by taxol (paclitaxel) or camptothecin treatment during colony formation in MCF-7 cells, suggesting that morphological changes could increase survival of cells treated with anticancer drugs. Thick circumferential bundles of microtubules around the periphery of the cells and chromatin condensation was not observed for MCF-7 cells on COL-I- and Matrigel-coated dishes treated with paclitaxel. To confirm this, spheroid cells were prepared, and we found that cytotoxicity was decreased for these cells, and significantly increased when cells were co-cultured on Matrigel- or COL-I-coated upper wells. The effect of anticancer drugs on cell survival was efficiently inhibited by interleukin-6 (IL-6) and interleukin-8 (IL-8).** ***Conclusions:*** **Present results suggested that not only integrin-ECM interactions but also other factors such as IL-6 and IL-8 secreted by cancer cells, cultured on COL-I and Matrigel dishes, are involved in the acquisition of drug resistance by MCF-7.**
Key Words: **spheroid cells, cell-cell adhesion, MCF-7, Caco-2, ECM, drug resistance.**

An extracellular matrix (ECM) is a complex network of adhesive proteins and proteoglycans, which regulates morphology, adhesion, proliferation, migration and differentiation of mammalian cells. It is well known that tumor cells interact with ECM components, such as laminin (LN), fibronectin (FN), and collagen type I (COL-I), via certain adhesion molecules such as integrins [1, 2]. Integrins are heterodimers consisting of α and β subunits and over 20 types of heterodimers have been identified to date [3]. In addition, ECM-integrin interaction stimulates signal transduction pathways that regulate a number of cellular processes in cancer growth, including cell cycle transition and protection from apoptosis [4]. Many investigators have reported that ECM consists of collagens, VN, LN, and ligands for integrins [5–7]. Moreover, integrins attach to ECM ligands and induce the stimulation of intercellular signals suggesting that integrins are, at least in part, involved in the prevention of cell death [8].

Taxol (paclitaxel) and camptothecin (also known as irinotecan) are plant-derived antitumor agents. These drugs have been widely used for treatment of ovarian, breast, stomach, and non-small cell lung cancers. The mechanism of the antitumor effect by taxol (paclitaxel) is believed to be antimitotics, specifically the promotion of the irreversible assembly of tubulin into microtubules [9]. Camptothecin has also been developed as a chemotherapeutic agent and has significant antitumor activity by inhibiting DNA topoisomerase I [10, 11]. Although both drugs administered possess potent antitumor activity, recent reports have shown that treatment of these drugs to patients with malignancies has resulted in multidrug resistance [12].

Of the mechanisms of drug-resistance, two major mechanisms have been predicted. One is believed to be an over-expression of multidrug resistance 1 (MDR1), which is a gene encoding P-glycoprotein [13, 14]. This membrane-bounded protein acts as an efflux pump resulting in the decrease of the drug concentration within the cells [13, 14]. The other mechanism is known to be tubulin mutations resulting in alterations of either the assembly or stability of microtubules [15, 16]. In this respect, Damiano *et al*. have reported drug resistance to antitumor agents upon cell adhesion to extracellular matrix proteins [17]. Their report suggests that cell-cell adhesion or some morphological changes may be involved in drug resistance of cancer cells.

In general, the expression of adhesion molecules on the cell surface is believed to mediate the cell-ECM interaction and signal transduction regulating the cell survival process [18]. However, there is still limited data concerning the relationship between drug resistance and the cell-ECM interaction.

This paper describes the effect of anticancer drugs on tumor cell-cell adhesion and the effect of ECM components on drug resistance of tumor cells. Our results are expected to facilitate research on the study of the acquisition of drug resistance and would provide new insight into the molecular mechanism of drug resistance

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Abbreviations used: BSA – bovine serum albumin; COL-I – collagen type I; ECM – extracellular matrix; FBS – fetal bovine serum; FN – fibronectin; LN – laminin; MEM – minimum essential medium; VN – vitronectin; XTT – 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide.

when exposed to anticancer drugs such as taxol (paclitaxel) and camptothecin.

MATERIALS AND METHODS

Chemicals. Camptothecin, paclitaxel (taxol) and XTT sodium salt were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available.

Cell culture. MCF-7 cells were obtained from the Japan Health Sciences Foundation (Tokyo, Japan). Caco-2 and NCI-H292 cell lines were purchased from American Type Culture Collections (Manassas, VA, USA). MCF-7 cells were maintained in a minimum essential medium (MEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 0.15% $NaHCO_3$, 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), 1 mM pyruvic acid sodium salt (MP Biomedicals, Inc., Solon, OH, USA), 0.001% insulin, 100 units/mL penicillin G and 100 μg/mL streptomycin. NCI-H292 and Caco-2 were maintained in RPMI 1640 (Sigma-Aldrich Co., St. Louis, MO, USA) containing 10% FBS and were grown at 37 °C in the presence of 5% CO_2. The cells were cultured in a humidified 5% CO_2 incubator at 37 °C, and were sub-cultured twice at week. The medium was regularly changed three times a week.

Cell culture on ECMs. 96-well plates (Corning Inc, NY, USA) were coated with 10 μg/mL COL-I (KOKEN, Tokyo, Japan), VN (KOKEN, Tokyo, Japan), Matrigel (BD Bioscience, San Jose, CA, USA), or FN (Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) and allowed to air dry overnight under UV irradiation. 10 μg/mL heat-denatured BSA (Fraction V, Sigma-Aldrich, St. Louis, MO, USA) in PBS was added into each culture well for 1 h at 37 °C to block the nonspecific binding site. Cells (2×10^3 cells/mL) were cultured on ECM pre-coated plates and incubated at 37 °C. After 24 h, camptothecin or taxol (paclitaxel) was added to the plates at concentrations of 10 nM, 100 nM, 1 μM or 10 μM and incubated for 72 h at 37 °C. The cell cultures were washed three times with PBS, and the cytotoxicity was assessed by XTT assay.

Three-dimensional cell culture. The method for three-dimensional culture was described previously [19]. Briefly, cells were cultured with Matrigel, and a collagen gel solution (0.5 mL) consisting of eight bed volumes of COL-I solution (KOKEN, Tokyo, Japan) concentrated by MEM and reconstituted buffer (0.05 M NaOH, 200 mM HEPES, and 260 mM $NaHCO_3$) were added to each well (50 μL/well) and incubated for 60 min at 37 °C. Cell suspension (2×10^3 cells/mL) containing 10% FBS was added to the wells and cultured. After 24 h, camptothecin or paclitaxel (taxol) was added to the wells and incubated for 72 h at 37 °C. The cells were randomly selected from each well and photographs were taken under a phase-contrast microscope [x 400].

Preparation of artificially aggregated cells. An MCF-7 cell suspension (2×10^3 cells/20 μL) was seeded on the lids of 10-cm diameter dishes (BD Biosciences, San Jose, CA, USA). To allow cell-cell aggregation, the culture was incubated for 24 h. After 24 h incubation, 20 μL of a medium containing one cluster of cells was supplemented with 80 μL of MEM containing camptothecin or taxol (paclitaxel), and subsequently incubated for 72 h.

Assessment of the* cytotoxicity *of anticancer drugs. The effects of paclitaxel and camptothecin on cell growth were assessed by XTT assay. 50 μL of XTT solution containing phenazine methyl sulfate and XTT was added to a 96-well culture plate and measured at 490 nm. The assay was repeated at least three times to confirm the results.

Measurement of cytotoxicity in spheroid tumor cells. Cytotoxicity of taxol (paclitaxel) and camptothecin toward spheroid tumor cells was determined by measuring the area occupied by spheroid cells. Three-dimensional cultured cells or aggregated cells were seeded onto culture plates and treated with taxol (paclitaxel) or camptotethcin as indicated in Tables 1, 2. After 72 h, the spheroid cell size was measured with a computer assisted image analyzer (Image Hyper II, Osaka, Japan). Cell growth was expressed as the percentage of growth of cells in the absence of taxol (paclitaxel) or camptothecin. Fifty percent inhibition of cell proliferation (IC_{50}) was calculated from the concentration-response curve.

Table 1. Effects of several components of ECM on cancer cell survival upon treatment with anticancer drugs

		Survival (%)				
		BSA	FN	VN	COL-I	Matrigel
Caco-2	Taxol	19.2 ± 0.5	24.5 ± 5.3	22.5 ± 5.1	49.7 ± 3.1*	30.7 ± 4.2*
	Campto-thecin	4.7 ± 0.4	4.6 ± 0.5	4.1 ± 0.5	84.0 ± 17.1*	55.9 ± 1.2*
MCF-7	Taxol	53.9 ± 7.7	45.3 ± 1.0	36.3 ± 0.8	82.0 ± 2.4*	87.3 ± 1.0*
	Campto-thecin	64.3 ± 5.0	42.8 ± 0.6	48.4 ± 5.1	114.2 ± 1.9*	100.6 ± 1.8*
NCI-H292	Taxol	31.9 ± 0.8	29.1 ± 1.8	34.5 ± 0.5	36.1 ± 1.2*	124.1 ± 5.7*
	Campto-thecin	35.4 ± 1.1	37.0 ± 0.2	34.1 ± 2.0	50.1 ± 0.7*	69.6 ± 4.8*

Notes: Caco-2, MCF-7, and NCI-H292 cells were seeded on VN-, FN-, COL-I-, matrigel-, or BSA-coated cultured plates, and incubated at 37 °C. After 24 h of incubation, the cultures were supplemented with 10 μM anticancer drugs and cultured for 72 h. Cell survival was measured by XTT assay. Data are expressed as mean ± S.E. (*$P < 0.05$ *vs* BSA). BSA, bovine serum albmin; FN, fibronectin; VN, vitronectine; COL-I, collagen type I.

Table 2. Effects of anticancer drugs on spheroidal cells

		IC_{50} (μM)	
		Normal Culture	Spheroidal Culture
MCF-7	Taxol (Paclitaxel)	1.62 ± 0.32	3.91 ± 0.60*
	Camptothecin	3.13 ± 1.24	27.06 ± 3.56*

Notes: Cell suspension or spheroidal cells of MCF-7 cells were seeded into the culture plate and supplemented with various concentrations of taxol (paclitaxel) or camptothecin. After 72 h incubation, the area of spheroidal cells was measured with an image analyzer. The IC_{50} values of cell proliferation were calculated from the dose-response curve. Data are expressed as mean ± S.E. of 3–6 independent experiments (*$P < 0.05$).

Morphological observation. MCF-7 cells were cultured on ECM-coated plates. After 24 h, morphological changes of the cells were observed with a phase contrast microscope (Olympus, Tokyo, Japan) after the cells were fixed with 2.5% glutaraldehyde in PBS.

Effect of anticancer drugs on monolayer cells co-cultured with MCF-7 colonies. Using a trans-well chamber (Costar, Cambridge, MA, USA), MCF-7 cells (4×10^4 cells/80 μL) were cultured and added to the

COL-I-, Matrigel-, or BSA-coated plates and the cells were maintained for 2 to 3 days at 37 °C. 10 μM of camptothecin was added to a lower well and incubated for 72 h. Cell growth inhibition was determined by XTT assay.

Immunofluorescence. The MCF-7 cells were cultured on the COL-I- or Matrigel-coated culture slides (BD Biosciences, San Jose, CA, USA) with or without paclitaxel for 24 h. The cells were fixed with 4% paraformaldehyde in PBS for 20 min and subsequently soaked in phosphate buffered saline (PBS) for 10 min. They were then permeabilized with 0.2% Triton X-100 in PBS for 10 min and blocked by PBS containing 3% BSA for 30 min at room temperature. Immunostaining was performed using monoclonal anti-β-tubulin clone TUB 2.1 antibody (1 : 200; Sigma-Aldrich, St. Louis, MO, USA). Fluoroscein isothiocyanate (FITC)-conjugated anti-β-tubulin monoclonal antibody (Sigma-Aldrich Co., St. Louis, MO, USA) was used for this experiment. Initial incubation was performed using PBS containing 3% BSA for 2 h at room temperature. Anti-mouse IgG antibody conjugated to fluoroscein isothiocyanate (FITC) (1 : 100; Sigma-Aldrich, St. Louis, MO, USA) was used for secondary detection and DAPI (1 : 5,000; Invitrogen, Carlsbad, CA, USA) was used for nuclear staining. The stained cells were examined using a laser scanning confocal microscope (KEYENCE, Osaka, Japan).

Effects of IL-6 and -8 on the* cytotoxicity *of anticancer drugs. MCF-7 cells (2×10^3 cells/mL) were cultured in on 96-well plates and incubated at 37 °C. After 24 h incubation, camptothecin (10 μM) with or without recombinant human IL-6 and IL-8 (Peprotech, London, UK) were added at the dose of 1 and 10 ng/mL to the wells and subsequently incubated for 72 h at 37 °C, then the cell viability was assessed by the XTT assay.

Statistical analysis. Statistical analysis was performed with the Bonferroni-Dunn procedure after ANOVA or the Mann-Whitney test. Data are expressed as the mean ± S.E. of each experiment ($P < 0.05$).

RESULTS AND DISCUSSION

It is known that cancer cells adhere to ECM via integrins, which contribute to the acquisition of drug resistance. To elucidate the role of cell adhesion in the process of acquisition of drug resistance toward antineoplastic agents, we studied the effect of taxol (paclitaxel) and camptothecin on three human cancer cell lines, human colon carcinoma cells (Caco-2), mammary breast cancer (MCF-7), and lung carcinoma cells (NCI-H292). These cell lines have been widely used for examining the effects of anticancer drugs. Cells were cultured on BSA-coated culture plates for 24 h to allow cell adhesion, and incubated with a medium containing taxol (paclitaxel) or camptothecin at different concentrations for 72 h. As shown on Fig. 1, the cell survivals were decreased in a concentration-dependent manner. However, we did not observe any significant differences in cell survival among these cell lines. Cell growth was significantly inhibited when cells were treated with camptothecin or taxol (paclitaxel).

The effects of ECMs (FN, VN, COL-I, and Matrigel) on cell growth inhibition by taxol (paclitaxel) and camptothecin were subsequently investigated. Table 1 shows the survival rates of Caco-2, MCF-7 and NCI-H292 cells cultured with various ECMs. As shown in Table 1, survival rates were higher when cells were cultured on COL-I- or Matrigel-coated plates compared with BSA. After treatment with camptothecin, MCF-7 cells grown on COL-I- and Matrigel-coated plates showed a marked increase of survival rate to 114.2% and 100.6%, respectively. Similar results have been observed when adding paclitaxel (taxol) to MCF-7. Thus, we found that COL-I and Matrigel promoted cell survival despite of anticancer drug treatment. However, we did not observe any significant changes of survival rates in all cell lines cultivated on FN- or VN-coated plates.

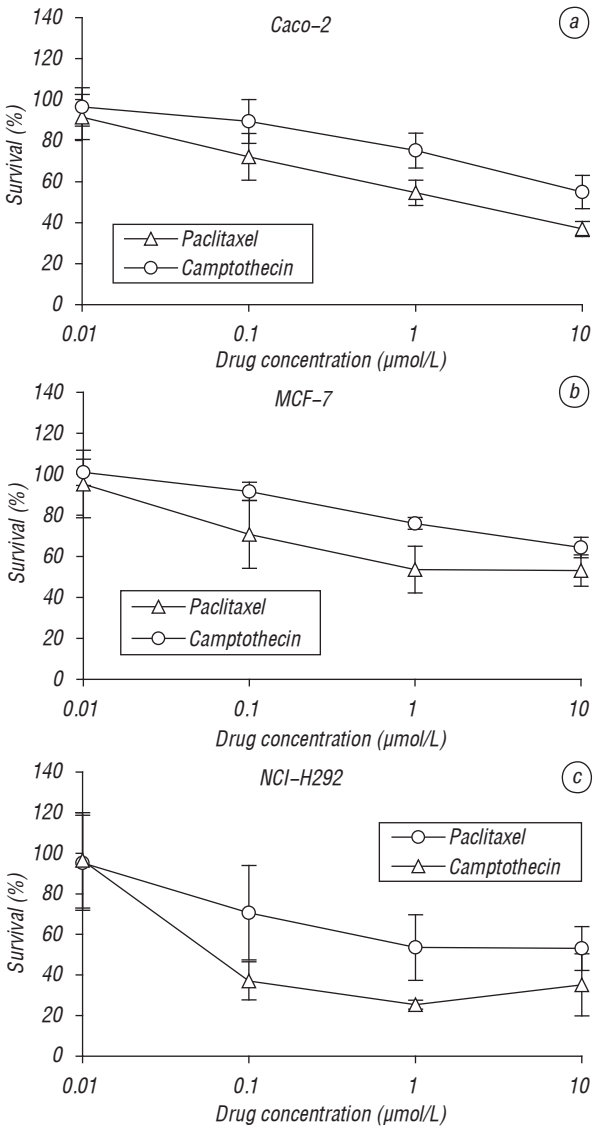

Fig. 1. Concentration-dependent inhibitory effects of anticancer drugs on cell proliferation. Caco-2 (*a*), MCF-7 (*b*), and NCI-H292 (*c*) cells were cultured on bovine serum albumin (BSA)-coated plates, and incubated at 37 °C for 24 h. After 24 h, camptothecin (○) or taxol (paclitaxel) (△) was added to the culture medium at various concentrations, and then cultured for 72 h. The inhibitory effect was measured by XTT assay. Cell survival rate is expressed as % of non-drug-treated cells

Cell-to-ECM adhesion is a crucial event in multi-cellular organisms for the modulation of cellular processes

such as growth and differentiation [20]. Particularly, regulation of anchorage-dependent processes is important for cell-ECM interaction [21]. Generally, the proliferation of many anchorage-dependent cell types is restricted to ECM-adhering cells [22, 23]. Lohi *et al.* [24] reported that collagen IV expression is increased in the tumor microenvironment. Except for FN and collagen, basement membrane ECM (Engelbreth-Holm-Swarm) suppressed apoptosis of CID-9 mammary epithelial cells and was induced by anti-integrin β_1 antibodies [25]. Cultured human endothelial cells retained viability by FN and VN treatment, and endothelial cell survival on VN was assumed to be mediated by integrin $\alpha_v\beta_3$ [26]. On the other hand, Chinese hamster ovary (CHO) cells can survive on FN but not on VN via integrin $\alpha_5\beta_1$ [27]. Thus, the role of cell-to-ECM interaction in survival depends on the cell line. These findings together with our previous results suggest that the cell-to-ECM interaction influences the survival of cells.

In the present study, the decrease of susceptibility to taxol (paclitaxel) and camptothecin was observed in the cells cultured on COL-I- and Matrigel-coated dishes (see Table 1). Since integrin β_1 is an inhibitor of apoptosis, our results suggested that integrin β_1 participated in the acquisition of resistance to taxol (paclitaxel) and camptothecin, and that these drugs are unable to affect cell growth when cells are cultured on COL-I and Matrigel.

Based on the results presented in Table 1, we subsequently investigated the morphological influence of ECMs (BSA, FN-, VN-, COL-I, and Matrigel) on MCF-7 cells. As shown on Fig. 2, MCF-7 cells were cultured on BSA (Fig. 2, *a*), FN (Fig. 2, *b*), and VN (Fig. 2, *c*) and formed a monolayer. Interestingly, MCF-7 cells when cultured on COL-I and Matrigel-coated dishes exhibited colony formation (Figs. 2, *d* and 2, *e*). However, colony formation was not observed for Caco-2 and NCI-H292 cells (data not shown). Previously, it was shown by Hirtenlehne *et al.* [28] that MCF-7 cells are able to form clusters when cultured on LN-coated plates. In addition, Kobayashi *et al.* [29] and Hoffman *et al.* [30] have reported that drug resistance has occurred when spheroid cells were cultured under *in-vivo*-like condition. Furthermore, Sakata *et al.* [31] reported that spheroid formation appeared to be more sensitive to drug resistance when compared with monolayer cultures. Based on their findings, the effect of morphological changes on drug resistance was proposed and further studied. We found that there was a correlation between morphological changes (spheroid formation) and decreased survival of cultured cells upon anticancer drug treatment (see Fig. 2, Table 1). Duan *et al*. [32] have shown that a three-dimensional culturing of MCF-7 cells could induce doxorubicin resistance. These authors suggested that cell-cell contacts could play a key role in the development of doxorubicin resistance. Larjava [33] have found that the interaction between ECM and integrin resulted in cytoskeletal rearrangements. Taking published results and our results into consideration, it could be assumed that the acquisition of drug resistance may be involved in morphological changes when cells are cultured with ECM components.

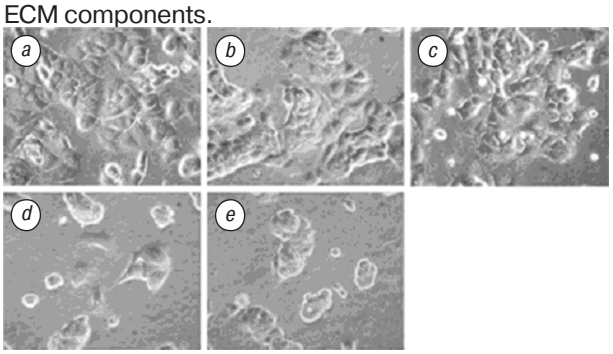

Fig. 2. Morphological changes of MCF-7 cells cultured on various types of ECMs. MCF-7 cells formed an island-shaped monolayer on BSA (*a*), FN (*b*) and VN (*c*), whereas cultured on COL-I (*d*) and Matrigel (*e*) exhibited colony formation. Other experimental conditions and details are described in the Materials and Methods section. *a–e*, x 400

To elucidate whether the morphological changes could affect cell survival, artificially aggregated MCF-7 cells (spheroid cells) were seeded and compared with cells grown in monolayers. The results are presented in Table 2. When MCF-7 cells were grown in monolayer, the IC_{50} values for taxol (paclitaxel) and camptothecin was 1.62 ± 0.32 μM and 3.13 ± 1.24 μM, respectively. On the other hand, when cells were grown as spheroid culture, the IC_{50} values for taxol (paclitaxel) or camptothecin increased by 2.4 times and 8.7 times, respectively. These findings indicated that drug resistance of spheroid-cultured cells was more significant than that of monolayer-cultured cells. Also, we could suggest that not only the cell-integrin-ECM interactions, but also cell-cell interactions could be involved in acquisition of drug resistance against some drugs by cancer cells.

It has been demonstrated that paclitaxel promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization [34]. Such stability results in the inhibition of the normal dynamic reorganization of the microtubule network required for vital interphase and mitotic intracellular functions [35]. On the basis of these findings, we decided to investigate the effect of paclitaxel, COL-I, and Matrigel on the paclitaxel-induced tubulin polymerization. The immunofluorescent staining of the microtubules' organization in MCF-7 cells cultured on BSA-, COL-I-, and Matrigel-coated plates without paclitaxel treatment is shown on Fig. 3 (*a–c*). As demonstrated on Fig. 3, *d*, the thick circumferential bundles of microtubules around the periphery of the cell and chromatin condensation was observed for MCF-7 cells on BSA plates with 1 μM paclitaxel. However, we did not observe similar effect of paclitaxel on MCF-7 cells cultured on COL-I- and Matrigel-coated plates (Figs. 3, *e* and 3, *f*). Ahmed *et al.* [36] have reported previously that loss of the ECM protein TGF-β1 (transforming growth factor beta 1) is sufficient to induce specific resistance to paclitaxel and mitotic spindle abnormalities in ovarian cancer cells. Our results suggest that COL-I and Matrigel induce paclitaxel resistance in MCF-7 breast carcinoma cells at the level of chromatin condensation and microtubules.

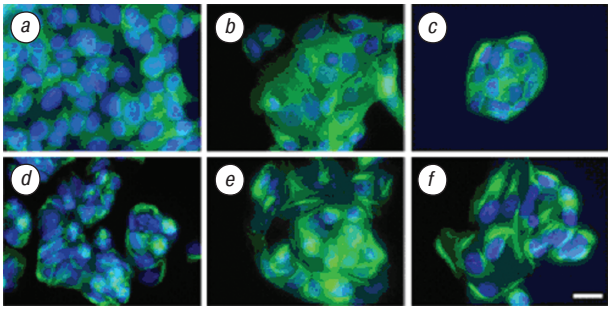

Fig. 3. COL-I and Matrigel induces paclitaxel resistance at the level of chromatin condensation and microtubules. Immunofluorescence micrograph exhibits the microtubule organization in a MCF-7 on BSA, COL-I, and Matrigel before the addition of paclitaxel (*a–c*). MCF-7 cells on BSA (*d*), COL-I (*e*) and Matrigel (*f*) were treated with 1 μM paclitaxel for 24 h. Other experimental conditions and details are described in Materials and Methods section. Green, tubulin; blue, DNA. Scale bar, 50 μm

Using a trans-well chamber, next we examined whether monolayer cells could acquire drug resistance. When MCF-7 cells were cultured on Matrigel- and COL-I-coated upper-wells, the cellular shape was spheroid. However, MCF-7 cells formed a monolayer on non-coated wells, but any apparent colony formation was observed (data not shown). Interestingly, the cell survival was increased when MCF-7 were cultured without ECM components (lower well) and co-cultured with Matrigel or COL-I (upper well) (Fig. 4), suggesting that certain soluble factors, such as IL-6 and IL-8 secreted from spheroid cells cultured in upper well, stimulated MCF-7 cells and promoted the cell survival in presence of anticancer drugs. Previously, Duan *et al.* [37] have reported that IL-6 and IL-8, known to promote cell growth and inflammation, were over-expressed in taxol (paclitaxel)- and doxorubicin-resistant cell lines. Although both IL-6 and IL-8 are overexpressed in taxol (paclitaxel)-resistant cell lines, IL-6 has a potential to contribute directly to taxol (paclitaxel) resistance in human osteosarcoma. To elucidate whether IL-6 and IL -8 affect the cytotoxicity of anticancer drugs in MCF-7 cells, we measured cell viability after treatment with camptothecin with or without IL-6 and IL-8. The results are presented on Fig. 5.

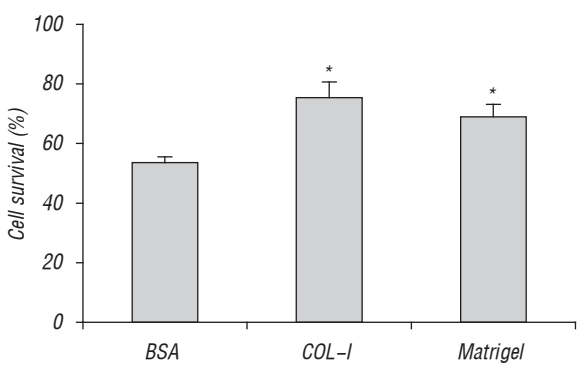

Fig. 4. Effect of camptothecin on monolayer MCF-7 cells co-cultured with MCF-7 colonies. MCF-7 cells were seeded on BSA-, COL-I-, and Matrigel-coated upper wells in trans-well chambers and then monolayer of MCF-7 cells were cultured in the lower chambers. 10 μM camptothecin was added to the lower chamber well and incubated for 72 h after the addition of camptothecin. Data are express as mean ± S.E.M. (*$P < 0.05$ *vs* BSA).

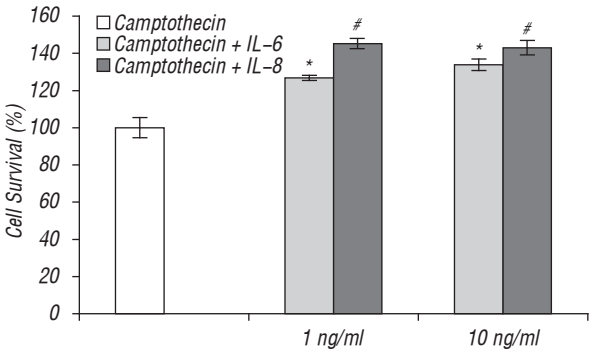

Fig. 5. Effects of Interleukin-6 and Interleukin-8 on the cytotoxicity of anticancer drugs. MCF-7 cells were seeded on 96-well plates. After 24h, 10 μM camptothecin with IL-6 (grey bar) or IL-8 (dark grey bar) were added to the wells and incubated for 72 h. Cytotoxicity was measured by XTT assay. Cell survival rate is expressed as % of camptothecin treated cells without Interleukins (open bar). Data are express as mean ± S.E.M. (*$P < 0.05$, $^{\#}P < 0.05$ *vs* non-interleukins-treated cells).

The survival rates of MCF-7 cells treated with 1 ng/mL IL-6 or IL-8 were 126.7 ± 1.35% and 133.8 ± 3.11%, respectively, when compared with non-treated cells. Moreover, the survival rates for MCF-7 cells treated with higher concentrations of IL-6 and IL-8 (10 ng/mL) were 145.2 ± 4.36% and 143 ± 6.55%, respectively. Similar results were observed for MCF-7 cells treated with 10 μM paclitaxel together or without IL-6 and IL-8 (data not shown). Therefore, our results suggest that IL-6 or IL-8 secreted by MCF-7 cells cultured on COL-I and Matrigel could contribute to the acquisition of resistance to paclitaxel (taxol) and camptothecin by MCF-7 cells. Considering our findings and already published data [41], we could conclude that not only integrin-ECM interactions, but cytokines secreted by spheroid cells (IL-6 and/or IL-8) may be important for the development of resistance to anticancer drugs by MCF-7 cells cultured on COL-I and Matrigel.

Obtained results provide new insight on mechanism of drug resistance acquired by spheroid cells exposed to anticancer drugs, and could facilitate further studies of mechanisms of drug resistance development.

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ВЛИЯНИЕ ИНТЕРЛЕЙКИНОВ НА ECM-ОПОСРЕДОВАННОЕ ПРИОБРЕТЕНИЕ ХИМИОРЕЗИСТЕНТНОСТИ КЛЕТКАМИ MCF-7

Цель: **изучить влияние различных компонентов внеклеточного матрикса (ECM) на приобретение химиорезистентности к таксолу и камптотецину клетками линии карциномы молочной железы MCF-7.** ***Методы:*** **клетки культивировали на платах, покрытых бычьим сывороточным альбумином (BSA), витронектином (VN), фибронектином (FN), коллагеном I типа (COL-I) или матригелем, без и с добавлением таксола (паклитаксел) или камптотецина. Влияние противоопухолевых препаратов на рост клеток изучали с помощью XTT-теста, изменения клеточной морфологии отмечали в фазовом контрастном микроскопе. Иммунофлуоресцентным методом определяли экспрессию β-тубулина.** ***Результаты:*** **для всех клеточных линий показано существенное снижение выживаемости после культивирования с противоопухолевыми препаратами без компонентов ECM, в то время как уровень выживаемости клеток Caco-2, MCF-7 и NCI-H292 значительно возрос при культивировании с таксолом или камптотецином в чашках, покрытых COL-I и матригелем. Для клеток MCF-7 показано формирование и сохранение колоний при культивировании в чашках с COL-I и матригелем. Более того, цитотоксичность (IC_{50}) таксола и во время колониеобразования клеток MCF-7 была снижена, что позволяет предположить, что морфологические изменения могут влиять на выживаемость клеток при культивировании с химиопрепаратом. Для клеток MCF-7, выращиваемых на чашках с COL-I и матригелем, не отмечали образования плотных периферических узлов микротрубочек и конденсации хроматина. Для подтверждения данного наблюдения проведены опыты с клетками, растущими в виде сфероидов. Показано, что цитотоксичность химиопрепаратов по отношению к этим клеткам снижалась и значительно повышалась при ко-культивировании с матригелем или COL-I в верхних камерах. Снижение выживаемости клеток под действием химиопрепаратов эффективно ингибировалось интерлейкином-6 (IL-6) и интерлейкином-8 (IL-8).** ***Выводы:*** **настоящие исследования показали, что не только интегрин-ECM-взаимодействия, но также и другие факторы, такие как IL-6 и IL-8, секретируемые опухолевыми клетками на чашках с COL-I и матригелем, участвуют в приобретении химиорезистентности опухолевыми клетками MCF-7.**

Ключевые слова: **сферические клетки, межклеточная адгезия, MCF-7, Caco-2, ECM, химиорезистентность.**